Psychoneuroendocrinology (2012) 37, 332—340

available at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/psyneuen

Symptom-specific associations between low cortisol responses and functional somatic symptoms: The TRAILS study

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Received 21 February 2011; received in revised form 27 June 2011; accepted 29 June 2011

KEYWORDS
Functional somatic symptoms;
Cortisol levels;
Adolescents;
Pain;
Fatigue

Summary
Background: Functional somatic symptoms (FSS), like chronic pain and overtiredness, are often assumed to be stress-related. Altered levels of the stress hormone cortisol could explain the association between stress and somatic complaints. We hypothesized that low cortisol levels after awakening and low cortisol levels during stress are differentially associated with specific FSS.
Methods: This study is performed in a subsample of TRAILS (Tracking Adolescents' Individual Lives Survey) consisting of 715 adolescents (mean age: 16.1 years, SD = 0.6, 51.3% girls). Adolescents' cortisol levels after awakening and during a social stress task were assessed. The area under the curve with respect to the ground (AUCg) and the area under the curve above the baseline (AUCab) were calculated for these cortisol levels. FSS were measured using the Youth Self-Report and pain questions. Based upon a factor analysis, FSS were divided into two clusters, one consisting of headache and gastrointestinal symptoms and the other consisting of overtiredness, dizziness and musculoskeletal pain.
Results: Regression analyses revealed that the cluster of headache and gastrointestinal symptoms was associated with a low AUCg of cortisol levels during stress ($\beta = -.09$, $p = .03$) and the cluster of overtiredness, dizziness and musculoskeletal pain with a low AUCg of cortisol levels after awakening ($\beta = -.15$, $p = .008$). All these analyses were adjusted for the potential confounders smoking, physical activity level, depression, corticosteroid use, oral contraceptive use, gender, body mass index and, if applicable, awakening time.
Conclusion: Two clusters of FSS are differentially associated with the stress hormone cortisol.

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0306-4530
doi:10.1016/j.psyneuen.2011.06.016

1. Introduction

Functional somatic symptoms (FSS), somatic symptoms which cannot be fully explained by underlying pathology, are very common during adolescence (Janssens et al., 2009). They are a burden for the child and the family (Hunfeld et al., 2002). Adolescents experiencing FSS frequently miss school (Konijnenberg et al., 2005; Roth-Isigkeit et al., 2005), and their symptoms ultimately contribute to high health care costs (Sleed et al., 2005). More insight into the etiology of this important health problem might aid the development of effective prevention and intervention strategies. FSS are thought to be the result of a complex interplay between biological, psychological and social factors, of which the latter are likely to be the most generic and the first the most symptom-specific risk factors. Indeed, social risk factors such as parental overprotection and peer victimization have been associated with FSS in general (Gini and Pozzoli, 2009; Janssens et al., 2009), whereas biological risk factors such as pubertal maturation (Janssens et al., in press) have been associated with specific symptoms.

Since FSS have often been found to be stress-related, the level of the stress hormone cortisol has often been investigated in relation to specific FSS (Tak and Rosmalen, 2010). Both low and high cortisol levels have been related to abdominal pain in adolescents (Alfven et al., 1994; Tornhage and Alfven, 2006), and low cortisol levels have been related to fatigue (Segal et al., 2005), but not all studies found significant associations between cortisol levels and fatigue (ter Wolbeek et al., 2007; Wyller et al., 2010). These divergent findings might be due to the small sample sizes used in most studies, which increased the risk of chance findings and false null findings. Cortisol studies are often underpowered (Tak and Rosmalen, 2010). Another explanation for these divergent findings is that the association with cortisol is symptom-specific. This explanation is in line with a recent meta-analysis in adults, which compared cortisol levels in healthy controls with those in patients with functional somatic syndromes, particularly chronic fatigue syndrome (characterized by overtiredness), fibromyalgia (characterized by musculoskeletal pain), and irritable bowel syndrome (characterized by gastrointestinal symptoms). This meta-analysis showed that low cortisol levels were found in chronic fatigue syndrome and fibromyalgia, but not in irritable bowel syndrome (Tak et al., 2011). These findings might suggest that biological pathways differ between fatigue and musculoskeletal pain on the one hand and gastrointestinal symptoms on the other hand. In accordance with this suggestion, we previously found in two cohorts of adolescents that pubertal stage is a risk factor for back pain, overtiredness, and dizziness, but not for stomach pain and headache (Janssens et al., in press).

Most previous studies examined whether cortisol levels under non-stressful conditions are related to FSS, most often by examining the cortisol awakening response (CAR). The CAR is the rapid cortisol increase during the first 30 min after awakening (Fekedulegn et al., 2007). It is a discrete and distinct component of the cortisol circadian cycle, with characteristics unrelated to those of cortisol secretion throughout the rest of the day (Clow et al., 2010). Interestingly, previous studies have found an association between low CARs and FSS (Roberts et al., 2004; Riva et al., 2010). However, it might be argued that cortisol levels under stressful conditions are closer related to FSS. Cortisol helps the body to adapt to stressful conditions by, among other things, increasing glucose levels and suppressing pain (Lariviere and Melzack, 2000; Seematter et al., 2004). Therefore, a blunted cortisol response will probably result in decreased energy supply and decreased pain suppression, which may ultimately result in FSS. Studies that examined whether cortisol levels under stressful conditions are truly related to FSS in adolescents are, to the best of our knowledge, lacking.

Thus, research on the relation between cortisol levels and FSS in adolescents is limited and findings are inconsistent. We hypothesized that the association between FSS and cortisol is symptom-specific: we expect an association of low cortisol with overtiredness, dizziness and musculoskeletal pain, but not with gastrointestinal symptoms and headache. Furthermore, we hypothesized that these associations are particularly present under stressful conditions, as opposed to cortisol levels under non-stressful conditions (i.e. after awakening). We examined our hypotheses in 715 adolescents from a general population cohort.

2. Methods

2.1. Participants

The data were collected in a subsample of TRAILS (Tracking Adolescents' Individual Lives Survey), a large prospective population study of Dutch adolescents with bi- or triennial measurements from age 11 to at least age 25. Thus far, three assessment waves of TRAILS have been completed, running from March 2001 to July 2002 (T1), September 2003 to December 2004 (T2), and September 2005 to December 2007 (T3). During T1, 2230 children were enrolled in the study (for more details about the sample selection, see de Winter et al., 2005), of whom 1816 (81.4%) participated in T3. During T3, 744 adolescents were invited to perform a series of laboratory tasks (hereafter referred to as the experimental session) on top of the usual assessments, of whom 715 (96.1%) agreed to do so. The costly and labor-intensive nature of the laboratory tasks precluded assessing the whole sample. To increase the power to detect mental health-related differences in stress responses, adolescents with a high risk of mental health problems had a greater chance of being selected for the experimental session. High risk was defined based on three criteria: temperament (i.e., high frustration and fearfulness and low effortful control) assessed by the revised parental version of the Early Adolescent Temperament Questionnaire at baseline (Ellis, 2002); lifetime parental psychopathology assessed by a parental interview at baseline; and living in a single-parent family also assessed by the parental interview at baseline (for more information see Bouma et al., 2009). In total, 66.0% of the focus sample had at least one of the above-described risk factors; the remaining 34.0% were selected randomly from the low-risk TRAILS participants. Please note that the focus sample still represented the whole range of problems seen in a normal population of adolescents, which made it possible to reproduce the distribution in the total TRAILS sample by means of sampling weights.

2.2. Procedure

The experimental session consisted of a number of different challenges, listed here in chronological order: a spatial orienting task, a gambling task, a startle reflex task, and a social stress test. The session was preceded and followed by a 40-min period of rest. The participants filled out a number of questionnaires at the start and end of the session. Before, during, and after the experimental session, extensively trained test assistants assessed cardiovascular measures, cortisol, and perceived stress. Measures that were used in the present study are described more extensively below. The experimental sessions took place in sound-proof rooms with blinded windows at selected locations in the participants' towns of residence. The total session lasted about three-and-a-half hour, and started between 0800 h and 0930 h (morning sessions, 50%) or between 1300 h and 1430 h (afternoon sessions, 50%). The protocol was approved by the Central Committee on Research Involving Human Subjects (CCMO). All participating adolescents gave informed consent.

2.3. The social stress test

The social stress test was the last challenge of the experimental session. The test involved a standardized protocol, inspired by (but not identical to) the Trier Social Stress Task (Kirschbaum et al., 1993), for the induction of mild performance-related social stress. Socio-evaluative threats are highly salient challenges for adolescents and are known to be effective activators of various physiological stress systems, particularly in combination with uncontrollability; that is, in situations when negative consequences cannot be avoided (Dickerson and Kemeny, 2004). The social stress test consisted of two parts. First, the participants were instructed to prepare a 6-min speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged on content of speech as well as on use of voice and posture, and ranked by a panel of peers after the experiment. The participants had to speak continuously for the whole period of 6 min. The test assistant watched the performance critically, and showed no empathy or encouragement. The speech was followed by a 3-min interlude in which the participants were not allowed to speak. During this interval, which was included to assess cardiac autonomic measures that were not affected by speech, the participants were told that they had to wait for a moment because of computer problems, but that the task would continue as soon as these problems were solved. Subsequently, during the second part of the social stress test, adolescents were asked to perform mental arithmetic. The participants were instructed to repeatedly subtract the number 17 from a larger sum, starting with 13,278. A sense of uncontrollability was induced by repeated negative feedback from the test assistant (e.g., ''No, wrong again, begin at 13,278''; ''Stop wiggling your hands''; ''You are too slow, we are running behind schedule''). The mental arithmetic challenge lasted for 6 min, again followed by a 3-min period of silence, after which the participants were debriefed about the experiment.

2.4. Measures

2.4.1. Cortisol

Cortisol levels were not only assessed during the stress experiment, but also on the morning before the stress experiment. Adolescents received a letter in which they were instructed to collect their cortisol at home immediately after awakening (CA1) and 30 min later (CA2). They were asked not to eat, brush their teeth or engage in heavy exercise during this 30 min. The area under the curve with respect to the ground (AUCg) and the area under the curve above the baseline (AUCab) of these morning cortisol levels were calculated (Fig. 1a and b, respectively). The first is a good indicator of the total amount of cortisol upon awakening. The latter is a good indicator of the CAR. For 35 adolescents the morning cortisol samples collected on the day of the experiment were missing or of insufficient quality; they were asked to collect their morning cortisol again on another day. Excluding cortisol samples that were collected on another day did not change our results. Adolescents (n = 18) who reported to have collected their first salivary cortisol sample more than 5 min after awakening, were excluded from our analyses. To calculate the AUCg of the morning cortisol levels we used the trapezoid formula proposed by Pruessner et al. (2003), that is, (CA1 + CA2)*30/2. The AUCab was calculated using the formula (CA2 − CA1)/2*30 for adolescents who showed a positive CAR and set at zero for adolescents who showed a negative CAR.

Cortisol levels during the experimental session were assessed in the lab by the test assistant just before the start of the social stress test (CS1), directly after the end of the test (CS2), 20 min after the test (CS3), and 40 min after the test (CS4). Considering the normal delay (20—25 min) in peak cortisol responses to experimental stressors (Kirschbaum et al., 1992), all measures reflected cortisol levels about 20 min earlier. Therefore, the measures reflected cortisol activity before, during and after the stress test. The AUCg of the cortisol stress levels (Fig. 1c) was calculated using the trapezoid formula: (CS1 + CS2)*25/2 + (CS2 + CS3)*20/2 + (CS3+CS4)*20/2. The calculation of the AUCab of the cortisol stress levels (Fig. 1d) was more complex, because it had to account for the possibility that cortisol levels dropped below baseline level (Appendix A).

Cortisol was assessed from saliva collected using the Salivette sampling device (Sarstedt, Numbrecht, Germany). After the experimental session, the samples were placed in a refrigerator at 4 °C, and within a few days stored at −20 °C until analysis. All samples were analyzed with the same reagent, and all samples from a participant were assayed in the same batch. Cortisol was measured directly in duplicate in 100 μl saliva using an in-house radioimmunoassay (RIA) applying a polyclonal rabbit cortisol antibody and 1,2,6,7 ^{3}H Cortisol (Amersham International Ltd., Amersham, UK) as tracer. After incubation for 30 min at 60 °C, the bound and free fractions were separated using activated charcoal. The intra-assay coefficient of variation was 8.2% for concentrations of 1.5 nM, 4.1% for concentrations of 15 nM, and 5.4% for concentrations of 30 nM. The inter-assay coefficients of variation were, respectively, 12.6%, 5.6%, and 6.0%. The detection border was 0.9 nM. Missing samples (C1: N = 12, C2: N = 8, C3: N = 10, C4: N = 12) were due to detection failures in the lab (60%) or insufficient saliva in the tubes (40%).

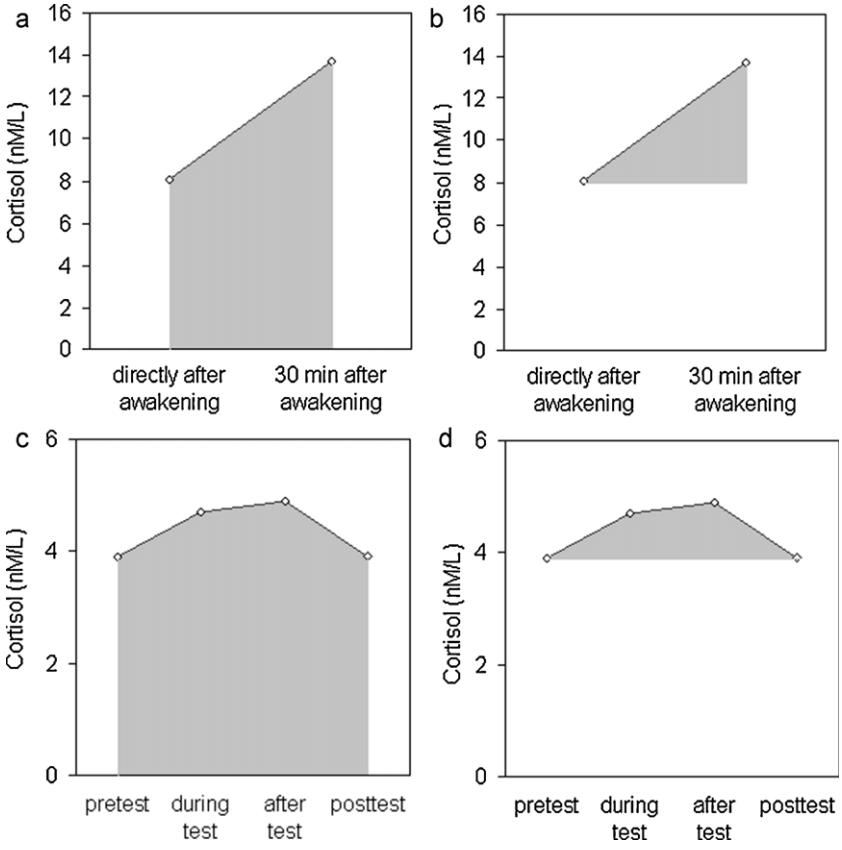

Figure 1 (a) Mean area under the curve with respect to the ground (AUCg) of cortisol levels after awakening. (b) Mean area under the curve above the baseline (AUCab) of cortisol levels after awakening, also known as the cortisol awakening response (CAR). (c) Mean area under the curve with respect to the ground (AUCg) of cortisol levels during stress. (d) Mean area under the curve above the baseline (AUCab) of cortisol levels during stress.

2.4.2. Functional somatic symptoms

FSS were measured by the Somatic Complaints scale of the Youth Self-Report (YSR) (Achenbach et al., 2003). This scale contains items referring to somatic complaints without a known medical cause or without obvious reason. The adolescents could indicate whether they experienced these complaints on a three point scale with 0 = never, 1 = sometimes or a little bit, or 2 = often or a lot. The items overtiredness, dizziness, headache, stomach pain, vomiting and nausea were used from this scale. Since the Youth Self-Report did not include musculoskeletal symptoms, those symptoms were assessed by asking participants questions about how often they experienced pain in their neck, back, shoulders, arms and legs during the past three months. Questions were rated on a 7-point measurement scale with response categories: 'Not at all', 'Less than once a month', 'Once a month', 'Two to three times a month', 'Once a week', 'Two to six times a week', and 'Almost every day'. A mean item score of the three gastrointestinal symptoms and of the five musculoskeletal symptoms was created. The mean item score of the five musculoskeletal pain symptoms was divided by three-and-a-half to rescale to the YSR.

We examined whether the symptoms could be divided into symptom clusters to diminish the amount of analyses, and thereby reduce the risk of chance findings. An exploratory factor analysis was performed with principal component extraction and oblimin rotation. Based upon our hypotheses a two-factor solution was requested. The factor analysis supported the division of symptoms into two clusters, one consisting of headache and gastrointestinal symptoms, and the other consisting of overtiredness, dizziness and musculoskeletal pain (Table 1). Moreover, a confirmatory factor analysis showed that this subdivision had excellent model fits ($\chi^2 = 3.6$ [df = 4], $p = .46$; Tucker-Lewis Index = 1.0). Mean item scores of the clusters were computed that did not take into account factor loadings, since factor loadings are sample-specific. Thus, for the first cluster the scores of headache

Table 1 Factor analysis to divide the functional somatic symptoms into two clusters.

	Factor 1	Factor 2
Headache	0.84	0.30
Gastrointestinal symptoms	0.84	0.26
Dizziness	0.52	0.58
Overtiredness	0.50	0.71
Musculoskeletal pain	0.11	0.84

Extraction method: Principal Component Analysis; Oblimin Rotation with Kaiser normalization. A two-factor solution was requested. The first factor had an eigenvalue of 2.20 (explained covariance 44%) and the second of 0.97 (explained covariance 19%).

and gastrointestinal symptoms were added and divided by two, and for the second cluster the scores of overtiredness, dizziness and musculoskeletal symptoms were added and divided by three.

2.4.3. Other variables

Gender, depression, body mass index (BMI), smoking, physical activity level, oral contraceptive use, corticosteroid use, and awakening time are known to be potential confounders in the relationship between cortisol and FSS (Likis, 2002; Rosmalen et al., 2005; Bouma et al., 2009; Janssens et al., 2009, 2010; Rimmele et al., 2009; Paananen et al., 2010; Tak et al., 2011) and were thus included in this study as covariates. Depression was measured using the Affective Problems scale of the Youth Self-Report (13 items, Cronbach's alpha = .75, see Janssens et al., 2010). Physical activity level and smoking frequency were assessed as part of the regular T3 measurements, which were filled out at school, on average 3.1 month (*SD* = 5.1) before the experimental session. Smoking was defined as being a daily smoker. The use of oral contraceptives and corticosteroid was assessed by means of a checklist on current medication use administered at the beginning of the stress experiment. Ninety-four adolescents (13.1%) of the subsample were on medication, of whom 80 (11.2%) used medication for medical conditions, 10 (1.4%) for psychological problems, and 4 (0.5%) for both psychological and medical problems. Six adolescents used corticosteroids for which we adjust in our analyses. Length and weight were measured by trained test assistants. BMI is defined as the weight in kilograms divided by the length in meters squared. Awakening time was reported by the adolescents.

2.5. Statistical analyses

Linear regression analyses were performed to examine whether a particular cluster of FSS was associated with the AUCg and the AUCab of the cortisol levels after awakening, as well as with the AUCg and AUCab of the cortisol levels during stress. The AUCs, which were normally distributed after natural log transformations, were used as outcome variables in all analyses. Depression, BMI, smoking, physical activity level, oral contraceptive use, corticosteroid use and, in case of cortisol levels after awakening, awakening time were included as covariates. The two FSS clusters were included in the model simultaneously, so their effects were adjusted for each other. To examine to which extent our findings were due to extreme cortisol levels, we repeated the analyses while excluding all outliers (mean $\pm$ 3*SD). Furthermore, we examined whether the results found in our subsample deviated from the results that would be found in the general population by repeating the analyses while using sampling weights to correct for the oversampling on adolescents with a high risk of mental health problems. Test results with two-sided *p*-values lower than .05 were considered statistically significant.

3. Results

3.1. Descriptive statistics

Characteristics of the sample, clusters of FSS, cortisol measures and confounders are shown in Table 2. Pearson correlations between the cortisol measures are shown in Table 3. Of

Table 2 Sample characteristics of participants of the stress experiment.

	Valid *N*	Mean (*SD*)/percentage
Age	715	16.1 (0.6)
Girls	715	50.9%
Habitual smoking	699	17.3%
Physical activity level[a]	695	3.3 (2.1)
Body mass index	696	21.3 (3.3)
Corticosteroid use	715	0.8%
Oral contraceptive use	358 (girls)	35.2% of girls
Depression[b]	695	0.3 (0.2)
Cortisol directly after awakening (nM/L)	600	8.1 (5.7) 8.9 (5.1)
	35[e]	
Cortisol 30 min after awakening (nM/L)	612	13.7 (7.9) 14.1 (7.4)
	32[e]	
Cortisol just before the stress test (nM/L)	698	3.9 (4.1)
Cortisol directly after the stress test (nM/L)	704	4.7 (4.0)
Cortisol 20 min after stress test (nM/L)	702	4.6 (3.9)
Cortisol 40 min after stress test (nM/L)	700	3.9 (3.4)
Cluster of headache and gastrointestinal symptoms[c]	680	0.39 (0.43)
Cluster of overtiredness, dizziness and musculoskeletal pain[d]	679	0.36 (0.37)

[a] Mean number of days a week on which at least 1 h physical active.
[b] Mean item score of depression which could range from 0 to 2.
[c] Mean item score of the cluster of headache and gastrointestinal symptoms which could range from 0 to 2.
[d] Mean item score of the cluster of overtiredness, dizziness and musculoskeletal symptoms, which could range from 0 to 2.
[e] Adolescents who were asked to collect their cortisol again, since the first cortisol assessment was missing or of insufficient quality; AUCg, area under the curve with respect to the ground; AUCab, area under the curve above the baseline.

Table 3 Pearson correlations between cortisol areas under the curve after awakening and during a social stress test.

	LNAUCg (awakening)	LN AUCab (awakening)	LN AUCg (stress)	LN AUCab (stress)
LNAUCg (awakening)	X			
LN AUCab (awakening)	.19**	X		
LN AUCg (stress)	.08	.06	X	
LN AUCab (stress)	.10*	.05	.41**	X

LN, natural logarithmic transformed; AUCg, area under the curve with respect to the ground; AUCab, area under the curve above the baseline; awakening, cortisol levels after awakening; stress, cortisol levels during social stress.
* $p < 0.05$.
** $p < 0.01$.

all adolescents, 74.4% reported having experienced a symptom of the cluster of overtiredness, musculoskeletal pain or dizziness at least once during the past six months, whereas 54.9% had experienced a symptom of the cluster of headache and gastrointestinal symptoms, but mean item scores of the clusters were comparable (Table 2). The AUCab of the cortisol levels after awakening correlated moderately with the AUCg of the cortisol levels after awakening, and the AUCab of the cortisol levels during stress correlated moderately with the AUCg of the cortisol levels during stress (Table 3). Cortisol levels after awakening were only marginally correlated with cortisol levels during social stress.

3.2. Cortisol levels during awakening and clusters of FSS

Regression analyses showed that none of the clusters of FSS was significantly related to the AUCab of the cortisol levels upon awakening (Table 4). The cluster of overtiredness, dizziness, and musculoskeletal pain predicted a lower AUCg of the cortisol levels after awakening, whereas the cluster of headache and gastrointestinal symptoms did not. When we repeated these analyses while excluding outliers or using sampling weights to correct for the oversampling on adolescents with a higher risk of mental health problems, the results remained essentially the same.

3.3. Cortisol levels during the social stress test and clusters of FSS

None of the clusters of FSS predicted the AUCab of the cortisol levels during social stress (Table 4). The cluster of headache and gastrointestinal symptoms was associated with a low AUCg of the cortisol levels during social stress, whereas the cluster of overtiredness, dizziness and musculoskeletal pain was not. Again, repeating these analyses while excluding outliers or using sampling weights yielded essentially similar results.

4. Discussion

This study suggests that a cluster of overtiredness, dizziness and musculoskeletal pain is associated with low cortisol levels after awakening, whereas a cluster of gastrointestinal symptoms and headache is related to low cortisol levels during psychosocial stress.

There are several important strengths of this study. One strength is that it examined the relationship between particular clusters of FSS and cortisol levels under stressful and non-stressful conditions in a large sample, which enlarged the robustness of our findings. Furthermore, the generalizability of the results is increased by using a subsample of a general population cohort. Since results were comparable when using sampling weights, our findings can be generalized to the general population. Studies performed so far often examined patients suffering from functional somatic syndromes. Studying only patients makes it hard to translate findings to the general population. A final strength of this study is that it examined adolescents. Studies that examined the relationship between FSS and cortisol in adolescents are rare, although it is known that most FSS start to develop during adolescence.

Several limitations to our study have to be mentioned as well. The first limitation is that we measured cortisol levels and responses under non-stressful and stressful conditions at only one occasion. Cortisol levels and responses

Table 4 Relationships between clusters of FSS and the area under the curve of cortisol levels after awakening and during stress.

	Cortisol levels after awakening		Cortisol levels during stress	
	LN AUCab[a]	LN AUCg[a]	LN AUCab[b]	LN AUCg[b]
Cluster of headache and gastrointestinal symptoms	−0.01 (0.88)	0.08 (0.11)	−0.07 (0.09)	−0.10 (0.03)
Cluster of overtiredness, dizziness and musculoskeletal pain	−0.09 (0.12)	−0.15 (0.008)	0.01 (0.81)	−0.03 (0.58)

[a] Adjusted for gender, body mass index, smoking, oral contraceptive use, corticosteroid use, physical activity level, depression, and awakening time.
[b] Adjusted for gender, body mass index, smoking, oral contraceptive use, corticosteroid use, physical activity level, and depression; clusters are simultaneously included as predictors of the AUCs and associations are therefore adjusted for each other. LN, natural logarithmic transformed, AUCab, area under the curve above the baseline, AUCg, area under the curve with respect to ground; bold numbers indicate significant effects.

fluctuate over time and depend heavily on individual circumstances (Hellhammer et al., 2007). In addition, only self-reported information about the adherence to the saliva collection instructions was available. It is good to note that excluding adolescents who showed a negative CAR, a potential objective indicator of non-compliance (DeSantis et al., 2010), from our analyses did not change our results. Furthermore, CARs might have been higher than usual due to the anticipation stress of the upcoming stress experiment. Measurement of cortisol on different days would have yielded more reliable results, but was not feasible given the large sample size. Another limitation of our study is that the data are cross-sectional, which precludes to draw conclusions about the direction of the associations. A longitudinal study design is needed to examine whether cortisol levels influenced the amount of FSS or vice versa. A final limitation is that the musculoskeletal pain questions did not explicitly state that the pain had to occur without obvious or medical reason. Therefore, part of the reported musculoskeletal pain might have been due to medically explained conditions, like sport injuries. However, we should be careful in distinguishing between medically unexplained and medically explained symptoms, since it perpetuates mind—body dualism and doctors often disagree about whether a particular symptom is medically unexplained or not (Dimsdale et al., 2009).

Our findings are in line with a meta-analysis in adults that showed that fibromyalgia and chronic fatigue syndrome are related to low cortisol levels, whereas irritable bowel syndrome was not (Tak et al., 2011). Thus, our study supports the before-mentioned assumption that overtiredness, dizziness and musculoskeletal pain result from another biological pathway than headache and gastrointestinal symptoms. An explanation for these different pathways might be that gastrointestinal symptoms and headache, which were associated with low cortisol levels under stressful conditions, are often transient symptoms of stress. On the other hand, overtiredness, dizziness, and musculoskeletal pain, which were related to low cortisol levels under non-stressful conditions, might be symptoms of exhaustion due to chronic or recurrent exposure to stress. However, this needs further exploration.

Our finding of a significant association between cortisol levels (i.e. the AUCg) and clusters of FSS, but not between cortisol responses (i.e. the AUCab) and clusters of FSS indicates that adolescents suffering from FSS have cortisol responses that show a normal pattern, but occur at a lower level. This is in keeping with two previous studies that found that patients suffering from chronic fatigue syndrome and patients suffering from fibromyalgia had lower morning cortisol levels than healthy controls but not different morning cortisol responses (Roberts et al., 2004; Riva et al., 2010).

Contrary to the common assumption that FSS are somatic manifestations of a depression, our study suggests that FSS have a distinct physiological etiology from depression. Namely, after adjusting for depression the associations between low cortisol levels and FSS remained significant. Moreover, a study at the first assessment wave of TRAILS showed that somatic symptoms of depression (i.e. sleeping problems and eating problems) are associated with high cortisol awakening levels, whereas we found FSS to be related to low cortisol awakening levels (Bosch et al., 2009). This supports our previous finding that although depression and FSS are closely related, they are not the same (Janssens et al., 2010).

Because of the observational and cross-sectional design of this study, we cannot draw conclusions about whether the administration of cortisol is helpful for adolescents suffering from FSS. We believe caution is warranted, since the found association were only small and clinical trials in adults have shown that administering cortisol to reduce FSS was only beneficial to a small number of patients (Cleare et al., 1999). Further biological research on the two identified symptom clusters of FSS is needed for the development of effective treatment for adolescents suffering from those symptoms.

Role of funding source

The sponsors did not contribute in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

Prof. Verhulst is a contributing author of the Achenbach System of Empirically Based Assessment, from which he receives remuneration. All other authors (Dr. Janssens, Prof. Oldehinkel, Prof. Ormel, Dr. Hunfeld, and Prof. Rosmalen) do not declare potential conflicts of interests.

Acknowledgements

This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314, and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005); the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), and the participating universities. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible. We would also like to thank Lineke Tak for her helpful comments on an earlier draft of this manuscript.

Appendix A

SPSS syntax to compute the AUCab of the cortisol levels during stress

Step 1: Compute AUCabA (AUCab between CS1 and CS2)
IF (CS2 ≤ CS1) AUCabA = 0.
IF (CS2 > CS1) AUCabA = ((CS1+CS2)*12.5) − (CS1*25).

Step 2: Compute AUCabB (AUCab between CS2 and CS3)
IF ((CS2 ≤ CS1) & (CS3 ≤ CS1)) AUCabB = 0.
IF ((CS2 ≥ CS1) & (CS3 ≥ CS1))
AUCabB = (CS2 + CS3)*10 − (CS1*20).
IF ((CS2 ≥ CS1) & (CS3 ≤ CS1)) AUCabB = (CS2 − CS1)
*((CS2 − CS1)/(CS2 − CS3))*10.
IF ((CS2 ≤ CS1) & (CS3 ≥ CS1))
AUCabB = (CS3 − CS1)*((CS3 − CS1)/(CS3 − CS2))*10.

Step 3: Compute AUCabC (AUCab between CS3 and CS4)
IF ((CS3 ≤ CS1) & (CS4 ≤ CS1)) AUCabC = 0.
IF ((CS3 ≥ CS1) & (CS4 ≥ CS1))
AUCabC = (CS3 + CS4)*10 − (CS1*20).
IF ((CS3 ≥ CS1) & (CS4 ≤ CS1))
AUCabC = (CS3 − CS1)*((CS3 − CS1)/(CS3 − CS4))*10.
IF ((CS3 ≤ CS1) & (CS4 ≥ CS1))
AUCabC = (CS4 − CS1)*((CS4 − CS1)/(CS4 − CS3))*10.

Step 4: Compute AUCab of the cortisol stress response
COMPUTE CSRAUCab = AUCabA + AUCabB + AUCabC.

Legend: AUCab = area under the curve above the baseline, CS1 = cortisol level 20 min before the stress test, CS2 = cortisol level during the stress test, CS3 = cortisol level just after the stress test, CS4 = cortisol level 20 min after the stress test.

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